REMARKS

A check for a two-month extension of time accompanies this response. Any fees that may be due in connection with the filing of this paper or with this application may be charged to Deposit Account No. 06-1050. If a Petition for Extension of time is needed, this paper is to be considered such Petition.

Claims 17-22 and 24-41 are pending in this application. Claims 18-22, 40 and 41 are allowable and claims 35-39 are objected to as depending upon a rejected base claim but would be allowable if they were amended as independent claims or to depend on an allowable base claim. Claims 35-39 are not so-amended herein, however, pending consideration of the remarks below. Claims 17, 22, 35 and 39 are amended herein. Claim 17 is amended for clarity, and claims 22, 35 and 39 are amended to avoid duplicative claims. No new matter is added. Claim 39 as amended depends upon claim 17.

The Rejection of claims 17, 31 and 33 under 35 U.S.C. §103

Claims 17, 31 and 33 are rejected as being unpatentablee udner 35 U.S.C. §103(a) over Nolan *et al.* (WO 00/34436) in view of Neves *et al.* (Bioconjug. Chem (2000) 11:51-55) because Nolan *et al.* teaches introduction of chromosomes of 1-10 megabases into cells and the use of fluorescently labeled chromosomes with FACS analysis to determine the number of cells. It also is alleged that Nolan *et al.* teaches practice of these methods in fibroblast and parenchyma stem cells. It further is alleged that, although Nolan *et al.* does not teach fluorescent labeling of cells prior to introduction, this teaching is supplied by Neves *et al.* It is alleged that Neves *et al.* teaches methods of covalently labeling DNA with a fluorescent label, transfecting the labeled DNA into cells and determining the efficiency of transfection by fluorescence microscopy. The Office Action concludes that one of ordinary skill in the art would have been motivated to have combined the teachings of the two references to fluorescently label a chromosome using the method of Neves *et al.* for the purpose of detecting transfection of a chromosome into a host cells as taught by Nolan *et al.* The Office Action alleges that because both references describe labeling DNA fluorescenttly, there is no reason to expect that one of ordinary skill in the art could not fluorescently label a chromosome using the method of Neves *et al.* for use in the transfection and detection in cells as taught by Nolan *et al.* This rejection is respectfully traversed.

Relevant Law

In order to set forth a prima facie case of obviousness under 35 U.S.C.§103: (1) there must be some teaching, suggestion or incentive supporting the combination of cited references to produce the claimed invention (ACS Hospital Systems, Inc. v. Montefiore Hospital, 732 F.2d 1572, 1577, 221 USPQ 329, 933 (Fed. Cir. 1984)) and (2) the combination of the cited references must actually teach or suggest the claimed invention.

The mere fact that prior art may be modified to produce what is claimed does not make the modification obvious unless the prior art suggests the desirability of the modification. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, In re Papesh, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963).

The Claims

Claim 17 is directed to a method for monitoring the delivery of a large nucleic acid molecule into a cell. The method includes the steps of (a) labeling the large nucleic acid molecule, then; (b) delivering the labeled large nucleic acid molecule into a cell; and (c) detecting the labeled large nucleic acid molecule in the cell by flow cytometry, fluorimetry, cell imaging or fluorescence spectroscopy, as an indication of delivery of nucleic acid molecule into the cells. Dependent claim 31 specifies particular cell types. Dependent claim 33 specifies an additional step of (d) determining the number of cells containing the label.

Differences Between the Claims and the Teachings of the Cited References

Nolan *et al.*

Nolan *et al.* teaches a method and apparatus for introducing a single chromosome into a cell and then using FACS to screen for and identify cells that have incorporated a single chromosome. The introduction of the chromosome into the cells is mediated by electrically induced fusion of an encapsulated chromosome and a cell. In other embodiments, the chromosome is introduced by methods that require acquisition of sufficient kinetic energy using a linear accelerator to introduce the chromosome. In other embodiments, a single charged chromosome is introduced using a linear accelerator to accelerate the chromosome through the plasma membrane. In other embodiments, the cell and chromosome is maintained in medium containing phenol red, and a laser is used to excite the phenol red causing formation of a transient hole through which the chromosome enters.

Nowhere in these discussions does Nolan *et al.* teach or suggest labeling the chromosome prior to treating the cells and chromosomes with laser light, electrical energy or a linear accelerator. After discussion of the manner in which a chromosome is introduced into the cell, Nolan *et al.* teaches the use of FACS to verify introduction of chromosomes into the cell. Labeling of the chromosome is part of the verification aspect of the method.

Nolan *et al.*, states on page 8, lines, 1 and 2, that the methods introduce a single chromosome into a cell "dovetailed" with FACS. On page 9, lines 25 *et seq.*, *after* discussing introduction of a chromosome into a cell, Nolan *et al.* states that there are numerous methods to determine the successful incorporation of a chromosome into a cell. All of these methods include a labeling step. All exemplified embodiments describe labeling subsequent to introduction into a cell Hence, reading this disclosure renders it clear that Nolan *et al.* does not contemplate labeling a chromosome prior to its introduction into cell. The labeling of the chromosome is part of the verification step..

Further, Nolan *et al.* does not teach or suggest delivering labeled large nucleic acid molecules of any size into a cell. Nolan *et al.* teaches methods for verifying introduction of the chromosome in the cell. All methods described for verification follow introduction of the a chromosome. For example, in one method identification of the inserted chromosome is achieved using chromomycin A3 and Hoechst 33258, which are known dyes for staining chromosomes in cells after introduction of the chromosome. The cells are then analyzed using FACS of cells containing the stained chromosomes.

Neves *et al.*

Neves *et al.* teaches the labeling of plasmid DNA, approximately 7 kb, with p-azido-tetrafluorobenzylamido-lissamine and with rhodamine nucleotides. Neves *et al.* teaches the introduction of these small labeled plasmids and the detection of labeled cells by fluorescence microscopy. Neves *et al.* does not teach or suggest methods for labeling large nucleic acid molecules. It also does not teach or suggest a method of introducing labeled large nucleic acids into cells, nor measuring delivery of labeled large nucleic acids. The plasmids that are labeled, introduced and detected by the method of Neves *et al.* are only about 7 kb (see Figure 1, page 53).

ANALYSIS

As noted above, in the consideration of obviousness, the mere fact that prior art may be modified to produce the claimed subject matter does not make the modification obvious

unless the prior art suggests the desirability of the modification. In re Fritch, 23 U.S.P.Q.2d 1780 (Fed. Cir. 1992); see, also, In re Papesh, 315 F.2d 381, 137 U.S.P.Q. 43 (CCPA 1963). In addtition, "a reasonable expectation of success is the standard with which obviousness is determined." MPEP 2141. In the instant case, the Examiner has failed to set forth a *prima facie* case of obviousness:

The combination of Nolan *et al.* in view of Neves *et al.* does not result in the the instantly claimed methods.

The combination of teachings of the cited references does not result in the instantly claimed methods, which include directed to labeling, introducing and detecting the delivery of large nucleic acid molecules. The claims are directed to methods in which large nucleic acid molecules are labeled, introduced and monitored.

The combination of teachings of the cited references fails to teach any methods that include a step of labeling *large* nucleic acids prior to introduction into a cell. For instance, Nolan *et al.* does not teach any methods in which the large nucleic acid molecules are first labeled and then the labeled large nucleic acid is introduced into the cell and detected. As noted above, the labeling step is part of the verification step. The methods taught by Nolan *et al.* include staining the chromosomes using the known dyes chromomycin A3 and Hoechst 33258 *after* the chromosome is introduced into the cell. Hence, Nolan *et al.* teaches introducing a chromosome into a cell and then verifying its introduction by methods including fluorescent labeling *after* the chromosome is delivered into a cell, as a means of verifying that the chromosome has been incorporated into the cell. There is no teaching or suggestion by Nolan *et al.* of any methods for labeling large nucleic acid molecules *prior* to delivery into a cell.

The Office Action alleges that although there is no suggestion in the Nolan *et al.* for labeling the nucleic acid prior to introducing it into the cell, such absence does not mean that the embodiment would not be clearly contemplated by the teachings of Nolan *et al.* It is alleged that because there is nothing in the teachings of Nolan *et al.* that limit it to post-transfection labeling, that such embodiment must therefore be included.

Applicant respectfully disagrees. The Examiner has provided no evidence that one of ordinary skill in the art would read Nolan *et al.* and conclude that Nolan *et al.* suggests labeling a chromosome prior to delivery. As noted, Nolan *et al.* explicitly teaches introducing the nucleic acid molecule, and THEN labeling the introduced molecule. Reading

a more generic disclosure into Nolan *et al.* requires using the instant application as a guide, which relies on the improper use of hindsight.

Further, there is no evidence in the record of art teaching methods for labeling large nucleic acid molecules in a manner that could be detected by "flow cytometry, fluorimetry, cell imaging or fluorescence spectroscopy, as an indication of delivery of the nucleic acid molecule into the cells." The only methods available for labeling large nucleic acid molecules, taught by Nolan *et al.*, are methods for labeling a chromosome *after* its introduction into a cell. Hence, one cannot infer that Nolan *et al.* suggests labeling large nucleic acid molecules prior to delivery, when no such methods are described in Nolan *et al.* nor in any art of record.

The Office Action contends that Neves *et al.* supplies the missing steps of labeling the nucleic acid and introducing labeled nucleic acid into a cell. Neves *et al.*, however, does not cure the defects of Nolan *et al.* As noted above, the instantly claimed methods are directed to the labeling, introduction and detection of *large* labeled nucleic acids. Neves *et al.* does not teach or suggest methods for labeling large nucleic acids. The methods taught by Neves *et al.* are for labeling to 7 KB plasmids, which are orders of magnitude smaller than the large nucleic acids delivered by the methods of the instant claims. There is no teaching nor suggestion in Neves *et al.* or in Nolan *et al.* of how to apply such labeling methods to a large nucleic acid molecule nor how to combine such methods with the methods taught by Nolan for introduction of chromosomes into cells.

The Office Action alleges that one of ordinary skill in the art would have been motivated to combine the references because "there is no reason to expect that one of ordinary skill in the art could not fluorescently label a chromosome using the method taught by Neves *et al*...." First, the Examiner's statement misstates the applicable standard. To predicate an obviousness rejection on the combination of references, there must be a reasonable expectation of success. MPEP 2143.02. Second, neither reference however singly or in combination, provides any reasonable expectation that large nucleic acid molecules could successfully be labeled prior to introduction into a cell. There is nothing in Neves *et al.* that would suggest its methods are compatible with the methods of Nolan *et al.* for delivering a chromosome into a cell.

For example, Neves *et al.* teaches the use of purified circular plasmid DNA for the plasmid labeling reaction. The purified DNA is mixed *in vitro* with label and illuminated at

365nm. The plasmids bearing 60-100 fluorophores per plasmid are then purified on ion exchange chromatography. Neves *et al.*, however, does not teach or suggest how such procedure would be adapted to large DNA molecules such as the chromosome delivered by the method of Nolan *et al.* Neves *et al.* does not teach how to purify large nucleic acid molecules such as the chromosomes of Nolan *et al.* such that they can be labeled by the *in vitro* method disclosed therein. Nolan *et al.* also does not provide any methods for isolating chromosomes for use in labeling methods *in vitro*. Hence, neither reference teaches how to provide suitable purified large nucleic acid molecules that can be labeled using the method taught by Neves *et al.* Additionally, Neves *et al.* states that a feature of the labeling reaction is to control the number of fluorophores linked to the DNA which is determinate of nucleic acid integrity (page 55, col. 1). Neves *et al.* does not teach or suggest how the labeling reaction would be modified to label large nucleic acids, *e.g.*, the illumination parameters, times and amounts of label, to control the fluorophore ratio and provide integrity for large nucleic acids. Nolan *et al.* also does not teach or suggest how the labeling method of Neves *et al.* could be applied to a large nucleic acid molecule. Therefore, the combination of teachings of the references fails to provide a method for labeling large nucleic acids prior to introduction into a cell.

The combination of teachings of reference also fails to provide a method includes the step of delivering labeling nucleic acid molecules into a cell in a manner that permits detection of the labels nucleic acid after delivery. For example, the methods of Neves *et al.* label small circular plasmids, whereas the chromosomes used in the delivery methods of Nolan *et al.* are not small circular molecules, they are large linear chromosomes. According to Neves *et al.*, linear molecules labeled with the method provided therein are more sensitive to degradation by DNases when introduced into cells, resulting in the release and detection of labeled nucleotides rather than the detection of the labeled nucleic acid molecule (page 54, col. 2). Hence, using the method of Neves *et al.* and applying it to the chromosome delivery method taught by Nolan *et al.* would not result in a method for detecting the labeled large nucleic acid molecule in the cell is an indication of delivery of nucleic acid molecule into the cells. Therefore, one of ordinary skill in the art would not have held an expectation of success for the combination of the two methods.

Significantly, the combination of reference also fails to provide a method in which labeled nucleic acid can be successfully delivered and detected because the labeling method

of Neves *et al.* **is incompatible with a delivery method** of Nolan *et al.* The labeling reaction taught by Neves *et al.* is activated by light and is light-sensitive (see for example col. 1 page 52 and col. 1 page 53). Additionally, Neves *et al.* states that "it is important to control the properties of the light source to maintain plasmid integrity" (page 54, col. 2). The method taught by Nolan *et al.* for introducing a chromosome into a cell *uses illumination in the form of a laser;* a light pulse from the laser is used to make a transient hole in the cell membrane and introduce the chromosome through that hole. Since the labeling reaction of Neves *et al.* is light-sensitive, the chromosome labeled by the method of Neves *et al.* is not suitable for use with the delivery method of Nolan *et al.*, which relies on a light pulse for delivery of the chromosome to a cell. Therefore, one of ordinary skill in the art would not have a reasonable expectation of success in the combination of the two methods. Furthermore, the combination of teachings cannot result in the instantly claimed methods, since a molecule labeled by the method Neves *et al.* **would not be delivered** by the method of Nolan *et al.*

Hence for all of these reasons, Applicant respectfully submits that the combination of Nolan *et al.* with Neves *et al.* does not teach or suggest, nor provide any reasonable expectation of success for a method that includes the steps of labeling large nucleic acid molecules, delivering large labeled molecules into a cell and detecting the labeled large nucleic acid molecule in the cell as an indication of delivery of nucleic acid molecule into the cells. Therefore, the Examiner has failed to set forth a *prima facie* case of obviousness.

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In view of the above, reconsideration of the above amendments and remarks and allowance are respectfully requested.

Respectfully submitted,

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